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Pericytes: Properties, Functions and Applications in Tissue Engineering

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Abstract Mesenchymal stem cells (MSCs) are one of the most studied adult stem cells and in recent years. They have become attractive agents/cell source for cellular therapy and regenerative medicine applications. During investigations about their origin, researchers hypothesized that perivascular regions are the common anatomical regions where MSCs come from and perivascular cells like pericytes (PCs) (Rouget cells, mural cells) are in vivo counterparts of MSCs. Beside capillaries and microvessels as their most common locations, PCs are also found in large vessels (arteries and veins). They can be isolated from several tissues and organs particularly from retina and brain. There are different approaches about their isolation, characterization and culture but there has been no common protocol yet because of the lack of defined PC-specific marker. They make special contact with endothelial cells in the basement membrane and have very important functions in several tissues and organs. They participate in vascular development, stabilization, maturation, and remodeling, blood pressure control, endothelial cell proliferation and differentiation, contractility of vascular smooth muscle cells, wound healing, vasculogenesis and angiogenesis, long-term maintenance of hematopoietic stem cells in bone marrow niche. Their multipotential differentiation capacity and participation in many events in the body make PCs preferred cells in tissue engineering applications including 3D blood–brain barrier models, skeletal muscle constructs, bone tissue engineering and tissue-engineered vascular grafts.

Keywords Mesenchymal stem cells · Pericytes · Isolation · Culture · Characterization · Tissue engineering · Vascular tissue engineering

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Introduction

Mesenchymal stem cells (MSCs) are one of the most studied adult stem cells that have self-renewal and multipotential differentiation capacity. They are originated from mesoderm layer and can differentiate into cells from mesodermal origin, such as adipocytes, osteocytes, chondrocytes, and myoblasts [1]. Firstly, they were isolated from rodent bone marrow (BM) as fibroblastoid cells that adhered to culture flask and formed colonies (colony-forming unit - fibroblast) in vitro by Friedenstein et.al. in 1960s [2, 3]. Later on, these cells were named as "mesenchymal stem cells" by Caplan [4].

Beside BM, MSCs are also isolated from adipose tissue [5], placenta [6], umbilical cord [7], umbilical cord blood [8], amniotic fluid [9], muscle [10], tendon [11], dental pulp [12], periodontal ligament [13], skin [14], in short almost all postnatal [15] and fetal tissues [16]. In 2006, Mesenchymal and *Tissue Stem Cell Committee of the International Society for Cellular Therapy* (ISCT) stated the minimum criteria for defining human MSCs: Cells must; 1) adhere to plastic, 2) express cell surface antigens CD105, CD73, and CD90, 3) not express the cell surface antigens CD45, CD34, CD14, CD11b, CD79α, CD19, or HLA-DR, 4) differentiate into osteoblasts, adipocytes, and chondroblasts in vitro [17].

In addition to their accessibility, ease of isolation / ex vivo expansion and multipotential differentiation capacity; MSCs

have several important functions that make them attractive therapeutic agents for cellular therapy and regenerative medicine. Release of trophic factors, immune modulation, migration to the site of injury or tumor microenvironment, and hematopoiesis support is the most important properties of MSCs in clinical settings [18]. They are more reliable and preferable than embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) for clinical applications because of the lack of ethical and safety issues [19]. Most common trials for MSC use in clinical settings are graft versus host disease (GVHD) following BM transplantation [20], orthopedic injuries [21], cardiovascular diseases [22], autoimmune diseases [23], liver diseases [24], and genetic diseases [25]. In recent years, MSCs are also very promising for use in tissue engineering applications, in which these cells are seeded in natural or synthetic scaffolds, in order to heal the diseased or damaged tissues and organs [26].

Although MSCs are searched so many years, there are some issues that have not been elucidated yet. For example, some researchers have investigated the anatomical location where MSCs originate from and *the* in vivo counterpart of them. They hypothesized that, MSCs can be isolated from all vascularized organs so perivascular region may be the common anatomical region, and perivascular cells may be a possible in vivo source of them [16]. Perivascular cells that include pericytes (PCs) and adventitial cells (ACs), have similar properties with MSCs like multipotential differentiation capacity (adipogenic, osteogenic, chondrogenic and myogenic differentiation), expressing the same cell surface markers (CD73, CD90 and CD105) and not expressing hematopoietic or endothelial cell surface markers or antigens (CD34, CD45, CD31 and von Willebrand factor) [27, 28]. According to these facts, Crisan et.al. showed that PCs are the in vivo counterparts and perivascular origin of cultured MSCs [16]. In this review, the properties and functions of PCs were emphasized then isolation, culturing and characterization methods are summarized. In addition, the most prominent areas of tissue engineering that PCs had been used were pointed out.

What Is a Pericyte?

Pericytes (also named as Rouget cells or mural cells) are perivascular cells that wrap around endothelial cells in capillaries and microvessels (*peri*: around, *cyte*: cell) [16]. In 1873, firstly Rouget described them as “non-pigmented adventitial cells” or “intramural PCs” [29] then German anatomist Zimmermann renamed these cells as “pericytes” in 1923 [29, 30]. Beside capillaries, arterioles and venules, PCs also reside at subendothelial region of large vessels [31, 32]. Morphologically PCs are fibroblast-like cells with prominent nucleus, little cytoplasm and they have several projections [33]. Most PCs are derived from mesoderm layer and others such as retinal and brain are derived from neural crest [33].

Small blood vessels are composed of endothelial cells (ECs), which are surrounded by basal membrane, and PCs (18). Larger blood vessels (arteries and veins) are composed of three layers; tunica intima, tunica media and tunica adventitia. Located in the interior of the vessel, tunica intima is comprised of ECs surrounding the vascular lumen. Elastic lamina separates tunica intima and media from each other. Tunica media, which is located in the middle of tunica intima and adventitia, is composed of smooth muscle cells. There is also elastic lamina between tunica media and adventitia. Tunica adventitia consists of fibroblasts and collagen fibrils located in the outer [34]. As a structural component of blood vessels, PCs are located between the tunica intima and media in the large vessels and around the endothelial layer of small vessels. They make special cell-cell contacts with ECs through holes on the basement membrane. Tight and gap junctions, N-cadherin and beta (β)-catenin-based adherence junctions together form these contacts that are named as peg-and-socket contacts [35]. Pericytes communicate with ECs by peg-and-socket contacts directly and by paracrine signaling [35, 36]. By this way, they control proliferation and differentiation of ECs and transmit the mechanical contractile forces to endothelium [36].

Beside direct contact, several signaling pathways that exist between PCs and ECs control the proliferation and differentiation of both cell types. Transforming growth factor β (TGF-β) / activin-like kinase receptor (ALK5) is one of these important signaling pathways. Latent TGF-β is synthesized by ECs and become activated by direct contact of ECs and PCs. Active TGF-β effects the proliferation of ECs through TGF-β receptor (TβR)/ALK-1/Smad5 pathway. TβR/ALK-5/Smad2-3 pathway provides differentiation of both PCs and ECs [37]. Another important pathways involved in PC-EC communication are angiopoietin 1 (Ang1) / receptor tyrosine kinase of the Tie family (Tie2) (a short surname of a receptor tyrosine kinase with immunoglobulin and epidermal growth factor homology domains-2) and Ang2/Tie2 pathways. PCs express Ang1 and effect ECs by interaction with Tie2 receptor on them. This interaction provides vessel stabilization and maturation. Ang2 (the antagonistic ligand of Tie2) is expressed mainly by ECs. Ang2/Tie2 signaling causes detachment and loss of PCs, which leads to vessel destabilization [38, 39]. Platelet derived growth factor b (PDGF-B) / PDGF receptor beta (PDGFR-β) signaling is crucial for expansion and migration of PCs along vessel during angiogenesis. PDGF-B is secreted by sprouting ECs during angiogenesis and it interacts with PDGFR-β on the PCs [40]. Sphingosine 1-phosphate (S1P) / endothelial differentiation gene (Edg or $S1P_1$) signaling is very important for stable and strong PC-EC contact. Peg-socket contacts include N-cadherin-based adherence junctions. N-cadherin–based endothelial–PC contacts are stabilized by the S1P/$S1P_1$ signaling pathway [41].

Pericyte to EC ratio differs from tissue to tissue according to the function, blood pressure and blood flow rate of the tissue [33, 42]. The retina and the brain have the highest ratio (1:1) among other organs for functional blood-retinal and blood–brain barrier. The ratio of skin and lung is 1:10 and the ratio of striated muscle is one of the lowest as 1:100. This ratio is dependent on blood pressure levels [43]. Larger vessels also have higher PC to EC ratio because of the hydrostatic pressure of the vessels [33].

Pericytes have multipotential differentiation capacity [16, 27]. Beside adipogenic, osteogenic and chondrogenic differentiation, in cases of vessel enlargement or remodeling they can differentiate into vascular smooth muscle cells [44]. In wound healing and inflammatory processes they can become a collagen type-I producing fibroblasts [45]. Pericytes can also differentiate into skeletal muscle cells [46, 47].

What Is the Importance of Pericytes?

During recent years PCs have gained increasing attention because they are important regulators of vascular development, stabilization, maturation and remodeling. They also participate in a) stabilization and control of permeability of blood vessels, b) blood pressure control, c) vasculogenesis and angiogenesis, d) immunological defense, e) coagulation, f) contractility and tone of vascular smooth muscle, g) physiological and pathological repair processes and also h) wound healing [48, 49]. With all of these functions PCs have very important roles in maintenance, physiological repair and regeneration of organs [50, 51].

Previously PCs were known as contractile cells because of the presence of microfilaments. Recently, it has been demonstrated that PCs secrete variety of vasoactive agents. They have been found to express alpha-smooth muscle actin (α-SMA), tropomyosin, myosin, cholinergic and adrenergic (α-2 and β-2) receptors so these muscle-like cells have been shown to regulate vessel diameter and blood flow [36, 52].

Another important property of PCs is their immunomodulatory effect similar as MSCs. They secrete several cytokines and chemokines that modulate inflammatory responses [52]. According to the study of Cheryl et.al. PCs are poorly immunogenic and have the ability to regulate CD4 T cell responses [53]. Like macrophages, PCs have phagocytic activity and express CR3 complement receptor, CD4, class I and II major histocompatibility complex molecules that are also macrophage markers [54]. By the Fc receptors, PCs can perform antibody-dependent phagocytosis. They express ligand-specific scavenger receptors [55].

Pericytes have specific functions in some organs such as brain, liver and kidney. Brain has the highest PC density because of the existence of blood brain barrier (BBB) that keeps the potentially toxic blood-derived factors away from brain cells [56, 57]. Pericytes participate in immunological defense in brain by performing macrophage-like activities. Studies have shown that they are precursors of macrophages in the brain [55]. By pinocytosis, small and soluble molecules are brought into the PCs and extracellular fluid of the brain is cleaned. In liver, PCs have specialized functions and they are named as hepatic stellate cells or Ito cells. They are less dense than PCs located in brain. They provide the exchange of metabolites between cells and the processing of toxins [58]. They remodel the extracellular matrix (ECM) of liver tissue by secreting ECM proteins and matrix metalloproteinases [59]. They also involved in vitamin A metabolism, hepatic tissue repair and in fibrotic responses to liver diseases [43, 60]. In kidney PCs are named as mesangial cells and are located at the glomerular capillaries. They form increased capillary surface area for increased blood ultrafiltration [61].

Pericytes are important residents of endosteal niche of hematopoietic stem cells (HSCs) in BM. Beside the perivascular reticular cells expressing C-X-C motif chemokine 12 (CXCL12) and perivascular nestin+ MSCs, PCs play role in maintenance and homing of HSCs and support their *stemness*. According to the study of Corselli et al. [62], nestin, CXCL12, and leptin receptor expressing human BM PCs are found to provide ex vivo long-term maintenance of HSCs by cell-to-cell contact and *Notch/ Jagged1* signaling [28].

Angiogenesis and Vasculogenesis

Pericytes have functions in vasculogenesis and angiogenesis beside their roles in vessel stability and maturation, blood flow regulation, immunomodulation and HSC maintenance,. Vasculogenesis is the development of blood vessels during third week of embryogenesis [63]. In vasculogenesis mesodermal precursors called angioblasts and EC precursors called hemangioblasts migrate to avascular areas and form primary vessel plexus [64]. At this stage, TGF-β1 stimulates the differentiation of PDGFR-β+PC progenitor cells. These cells are also affected by PDGF-B that is secreted by ECs in the capillary plexus [36].

Angiogenesis is the formation of new blood vessels from preexisting ones. After vasculogenesis, primary vessel plexus is remodeled and become functional by angiogenesis that includes endothelial sprouting, bridging and intussusception [63]. During vessel sprouting, first ECs are stimulated by angiogenic factors like vascular endothelial growth factor (VEGF) and then they degrade the extracellular matrix around them with the proteases they secrete [36]. Proliferating and migrating ECs form a new lumen-containing vessel. Then, this immature vessel is surrounded by PCs, which are attracted by the signals like PDGF-B, S1P-1 and angiopoietins secreted by ECs [61, 65]. These interactions between ECs and PCs provide proliferation and recruitment of PCs to newly formed

vessels. By this way, PCs support the vessel maturation and stabilization and transfer angiogenic signals along the vessel length [35]. Finally, PCs undergo morphological differentiation according to the needs of the specialized tissues they reside. For example, PCs in the brain become elongated and have multiple cytoplasmic processes for maintenance of BBB. On the other hand PCs in the kidney become compact and rounded for more blood ultrafiltration [66].

Important Challenges with Current Techniques: Isolation, Culture and Characterization

Isolation of Pericytes

Pericytes have been mostly isolated from bovine retina or brain where the PC to EC ratio is at the highest level [67]. The other alternative tissues and organs that have been preferred to isolate PCs are; skeletal muscle, adipose tissue, skin, fetal tissues like placenta and umbilical cord, BM, kidney and liver [68, 69]. Most of these tissues are not practical for routine studies (except fetal tissues) because they require invasive sampling procedures and are very scarce. In general, PC isolation protocols start with mechanic and enzymatic digestion of tissue material. At mechanic digestion procedure, vessels are separated from surrounding tissue and become ready for enzymatic digestion procedure. Generally, collagenase is used for enzymatic digestion [70, 71]. After enzymatic digestion, the digested suspension can be passed through a 100 or 40 μm mesh filter to remove large vessel segments and fibrous tissue. Then, it is followed by vessel outgrowth or positive immunoselection (by fluorescence-activated cell sorting (FACS) or magnetic-activated cell sorting (MACS)) according to CD146 marker. Primary vessel cultures often result with mixed population of cells. On the other hand, FACS and MACS procedures are more complicated and they often result with low numbers of cells [72].

For PC isolation, fetal tissues seem to be very promising compared to others because they are not invasive (often discarded at birth), easy to be reached, highly vascularized and are known to contain high amount of stem/progenitor cells. Chen et.al. isolated PCs from human placenta by mechanic and enzymatic dissociation of chorionic villi [47]. Then, using FACS they purified PCs. In another study Meier et.al. obtained human placenta from healthy full-term infants and they isolated PCs again by mechanic and enzymatic procedures [72].

Human umbilical cord (UC) is generally known as an abundant source of ECs and MSCs from Wharton's jelly. But recently UC vein has been demonstrated as an important source of perivascular cells and accordingly PCs that are members of them. Tsang et.al. isolated perivascular cells around the umbilical cord vessels [70]. First, they dissected the blood vessels and made sutures at both ends of the vessels. Then, they put the tied vessels into 1 mg/ml of collagenase solution and incubated them for 16 h at 37 °C. After collecting the digested cells and cultured them to passage two, they isolated CD146+ cells by using MACS. Hosseini et.al. isolated perivascular cells by performing the same procedure but before culturing the isolated cells, they performed CD45 depletion protocol by MACS in order to remove the hematopoietic cells [73]. In order to isolate perivascular cells, Schugar et.al. performed three different isolation techniques; 1) mechanical dissociation and explant culture technique, 2) enzymatic digestion with dispase, and 3) enzymatic digestion with collagenase [74].

Culture of Pericytes

Characteristics of pericytes have been defined by many researchers. According to Crisan et al., PCs do not attach to surface rapidly and divide very slowly especially up to fourth passage [29]. They can reach 80 % confluence within three to 4 weeks but do not reach 100 % confluence [75]. They should be feeded every 3 days and cultured at 37 °C in a humidified atmosphere containing 5 % CO_2. Morphologically, PCs are seen as large, irregular stellate shaped cells on the culture flask [29].

Researchers use differently formulated culture medias for culture of PCs. For example in the study of Nakagawa et.al., rat cerebral PCs were cultured on uncoated dishes by using DMEM supplemented with 10 % fetal bovine serum (FBS) [76]. Tsang et.al. cultured isolated perivascular cells in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 15 % embryonic stem cell qualified-fetal bovine serum (ESQ-FBS) [70]. Montemurro et.al. used different culture medium containing DMEM high-glucose, supplemented with 20 % FBS for feeding the cells [71]. Hosseini et.al. cultured perivascular cells in 75 % α-MEM, 15 % FBS [73]. At the study of Tigges et.al., the PCs isolated from mouse brain have been cultured in EC growth medium for two passages on freshly collagen-coated culture plates [77]. After the third passage cells were cultured in PC medium. In another protocol, pigment epithelium–derived growth factor (PEDF) has been added to the PC growth medium that includes also DMEM low-glucose, 10 % fetal bovine serum [78]. PEDF suppresses EC growth and promotes PC proliferation [79]. Blocki et.al. propagated human placenta-derived PCs in PC growth media [80]. It has been concluded that all of these mediums offer special areas for PC growth but it is clear that there is not a unique culture media for these cells.

Characterization of Pericytes

There is still a challenge about isolation and characterization of PCs because a general pan-PC molecular marker has not

been defined yet. However, there are some dynamic molecular markers for identification of PCs. These markers show different expression patterns and may be down or up-regulated according to anatomical location of PCs, developmental or angiogenic stage of blood vessel and in vitro culturing [66]. The most common markers that are used to identify PCs are CD146, PDGFR-β, regulator of G protein signaling 5 (RGS-5), α-SMA and neuron-glial 2 (NG2) [29, 81]. As a member of immunoglobulin superfamily and a transmembrane glycoprotein which functions as a Ca^{2+}-independent cell adhesion molecule, CD146 is a perivascular and EC marker which is expressed on vascular endothelium, PCs and smooth muscle cells [16, 29]. CD146 is also found on sinusoidal PCs in adult BM and gives their self-renewal capacity [82]. All PCs express CD146 so this marker is used to isolate PCs from heterogeneous cell suspensions obtained from human tissues [16]. PDGFR-β is one of the most frequently studied molecules expressed by PCs. It is also expressed by fibroblasts and astrocytes [36]. RGS5 is a member of "regulator of G protein signaling (RGS)" family that includes more than 25 GTPase-activating proteins [36]. It is expressed on activated PCs during vessel remodeling and tumor development [35]. α-SMA is universal marker of smooth muscle cells but it is also expressed on PCs and is associated with their contraction and function of controlling the blood pressure [83]. Neuron-glial 2 (also called high-molecular-weight melanoma-associated antigen) expression is seen on the surface of PCs during vasculogenesis and angiogenesis [36]. Differential expression of NG2 / α-SMA markers determines the subsets of human PCs as capillary-associated (NG2+ αSMA-), venule-associated (NG2-αSMA+) and arteriole-associated (NG2+αSMA+) PCs [36]. The other markers that are present on PCs are microtubule associated protein (MAP1B or 3G5), epidermal growth factor receptor, adenosine A_2 receptors, desmin, aminopeptidase A and N [29, 35, 81, 84].

Pericytes are most commonly characterized by observing positive expression of perivascular markers (CD146, PDGFR-β, NG2, α-SMA and RGS-5) and MSC markers (CD44, CD73, CD90, and CD105). They are negative for endothelial and hematopoietic markers (CD34, CD31, and von Willebrand factor) and myogenic and neural cell marker CD56 [29, 33, 81, 85]. By these expression patterns, PCs can be separated from other perivascular cells like adventitial cells that are negative for CD146 and positive for CD34 [85]. They are also characterized by showing their osteogenic, adipogenic and chondrogenic differentiation [28]. Immunocytochemical analysis is also used to identify PCs as their positive expression of NG2, α-SMA, 3G5 and negative expression of Chemicon, Calponin and CD31 [72].

Pericytes in Tissue Engineering

Tissue engineering (TE) was first defined in 1993 by Langer and Vacanti as "*an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ*" [86]. Since then, by the help of TE, clinicians have had alternative therapies for damaged tissues or organs that have limited repair/regenerative capacity and researchers have had three dimensional (3D) tissue/organ models for drug development and testing in vitro. Tissue engineering applications consist of; scaffolds for providing proper 3D shape of tissue construct and structural support; cells for forming tissues in vitro/within the body and growth factors for signaling and determining cell fate. The cells used in tissue-engineered constructs proliferate and differentiate on the scaffolds and secrete ECM proteins [87]. At first, differentiated cells were used for tissue engineering studies but with the development of the stem cell field, these studies have been relied on stem cells, including ESCs, MSCs, fetal stem cells (umbilical cord/placenta – derived stem cells) and iPSCs [88]. In addition to these stem cell types, recently pericytes have become cell sources for TE applications [89]. Pericytes have crucial roles in BBB function, blood vessel function/stability, angiogenesis and EC proliferation/differentiation. They can be isolated from fetal and adult tissues and have multipotential differentiation capacity as MSCs. All of these properties make PCs as preferred cells in the field of TE. The TE studies that include pericytes as cell sources were listed and summarized in Table 1.

Generally the drug development studies are carried on by using 2D cultures in vitro and by animal models. Current developments have shown that tissue-engineered 3D systems including multiple cell layers (or types) and a supporting biological matrix represent the in-vivo environment better than those monolayers on plastic dishes. Tissue engineered models are also more ethical and cheaper systems than animal models for drug screening studies. Blood brain barrier is one of the most studied structures for new therapeutic opportunities. Constructing a tissue-engineered model that best mimic BBB is important for drug discoveries. Pericytes can be used at these constructs because they are the natural residents of BBB. There is a study in which pericytes were used for constructing tissue-engineered BBB models and it is indicated in Table 1. In this study, Tourovskaia et.al. placed human brain PCs and astrocytes in a 3D hydrogel matrix containing collagen type I and succeeded to form BBB model by seeding ECs later in the natural scaffold [90]. They observed that PCs and astrocytes had located along the vessel and made contact with ECs tightly.

Pericytes can be isolated from adult human skeletal muscle and they have common properties and functions with skeletal muscle cells. Their function of supporting muscle

Table 1 Tissue engineering studies that include pericytes as cell sources

Pericyte source	Co-cultures	Scaffold type	Tissue engineered model	Study type	Result(s)	Year	Ref.
Human brain	- Human umbilical vein endothelial cells (HUVEC) - Human brain astrocytes	- Tubular chips (Polydimethylsiloxane (PDMS) halves, silicone septa, and microfibers) - Collagen type I gel	- Angiogenesis - The blood–brain barrier (BBB) - Tumor-cell extravasation	In vitro	- EC–pericyte interactions stimulated basement membrane formation - Pericytes were recruited to developing endothelial sprouts - The barrier function of the engineered microvessels was intact (The average permeability through the microvessel wall was 1×10^{-6} cm/s)	2014	[90]
Piglet and pig skeletal muscle	-	Polyethylene glycol (PEG)-fibrinogen based hydrogel scaffold (PF)	Skeletal muscle	In vitro In vivo	Pericyte–PF constructs promoted skeletal muscle regeneration and blood vessel growth (30 days in vivo)	2014	[91]
Human lipoaspirate	Human lipoaspirate adventitial cells (ACs)	Apatite-coated poly (lactic-coglycolic acid) (PLGA) scaffold	Bone tissue	In vivo	Healing of mouse critical-size calvarial defects (up to 61.5 and 69 % healing at 4 and 6 weeks, respectively)	2012	[92]
Human lipoaspirate stromal vascular fraction (SVF)	Human lipoaspirate SVF ACs	Ovine demineralized bone matrix putty	Intramuscular ectopic bone	In vivo	-hPSCs form bone in vivo (in 4 weaks) -hPSC-treated implants show an increase in vascularization	2013	[93]
Human brain	HUVEC	Fibrin gel	Microvasculature	In vitro	- Aligned microvascular networks form under defined conditions (at day 3) - Achieve high levels of microvessel alignment	2013	[94]
Human placenta	- HUVEC - Human lung fibroblasts - Human promyelocytic leukemia cells	PDMS microfluidic devices	Microvasculature	In vitro	- By day 4, pericytes were found adjacent to the blood vessels to cover abluminal surface of the endothelium. - Growth of microvascular networks that are similar in 3D architectures, intact barrier function, long-term stability and biochemical markers to their in vivo counterparts (at 4–5 days).	2013	[95]
Human skeletal muscle	HUVEC	Matrigel	Microvasculature	In vitro In vivo (direct injection of PCs)	2D and 3D Matrigel cultures/co-cultures using pericytes and HUVECs resulted with capillary-like structures within 6–12 h.	2013	[96]
Human placental microvasculature	HUVEC	Alginate beads (PCs) Protein gel (HUVEC)	Vascular tissue construct for paracrine signaling	In vitro In vivo	- Influence of HUVEC behavior - Encapsulated pericytes enhanced the formation of vessel-like structures (in 7 days) - Modulated the process of vascular self-assembly	2013	[97]
Skeletal muscle	-	Bi-layered elastomeric poly (ester-urethane) urea scaffolds	Vascular graft	In vivo	- After culturing for 2 days in a spinner flask, small diameter tissue engineered vascular graft was formed. - Patency of the vascular graft as an arterial conduit was maintained.	2010	[98]

Table 1 (continued)

Pericyte source	Co-cultures	Scaffold type	Tissue engineered model	Study type	Result(s)	Year	Ref.
Human BM-derived MSCs	HUVEC	Cell sheets	Vascularized osteogenic tissue	In vitro In vivo	- In vitro pre-vascularization of TE constructs - Promotion of a stable and mature supplying vasculature in vivo in 7 days.	2012	[99]
Human ESCs	HUVEC	- Rat tail collagen I - PDMS microfluidic channel	Microengineered three-dimensional vascular structures	In vitro	- Demonstration of 'blood vessel-on-a-chip' microfluidic assay recapitulates multiple aspects of in vivo angiogenesis - Demonstration of the potential for human embryonic stem cell-derived cells to be used in the engineering of organs-on-chips	2013	[100]
Bovine retina	Bovine brain and rat brain ECs	Polyhydroxymethylsiloxane (PHMS) surfaces	Microvasculature	In vitro	- Enhanced ECs growth in the co-culture system (12 h – 5 days) - Formation of microvessel-like structures (at 48 h)	2012	[101]
10 T1/2 cells (American Type Culture Collection)	HUVEC	Platelet-derived growth factor BB (PDGF-BB) coated PEG hydrogel	Microvasculature	In vitro In vivo (mouse cornea micropocket angiogenesis assay)	- Co-culture of cells resulted in tubule formation independent of surface modifications (within 6 days). - Bioactive hydrogels containing the combination of both soluble and immobilized PDGF-BB exhibited a significant increase in vessel density in angiogenesis assay.	2011	[102]

differentiation and angiogenesis makes PCs an attractive source of stem cell-mediated tissue-engineered muscle regeneration approaches [103]. There is also study in which pericytes were used for tissue-engineered muscle tissue and it is given in Table 1. In this study, Fuoco et al. seeded adult skeletal muscle-derived PCs on polyethylene glycol-fibrinogen-based scaffolds and generated a vascularized muscle construct for repair of ischemic or wounded muscle tissue [91].

Perivascular stem cells are new cell source for bone tissue engineering (BTE) because they have osteogenic differentiation capacity in vitro and after intramuscular implantation they can form bone tissue [92, 104]. U.S. Food and Drug Administration also approved their usage because of their potency, safety and purity [104]. The studies that consist of pericytes as the cell source for BTE were reported in Table 1. James et al. isolated human PCs from lipoaspirates and seeded them to apatite-coated poly lactic-coglycolic acid scaffolds. After implantation of this construct to mouse bone injury model, they proved the bone healing capacity of PCs [92].

Mendes et al. used osteogenic, endothelial and CD146+ cells and cell sheet technology-based constructs to provide in vivo vascularization of engineered construct for bone repair [99]. Cell sheets of perivascular-like cells and human umbilical vein ECs (HUVECs) are co-cultured on human BM MSC-derived osteogenic cell sheet. At this study, 3D cell sheet-based construct was obtained and by taking advantage of the interaction between CD146+ cells and HUVECs, vascularization of the tissue construct was accelerated [99].

Vascular tissue engineering (VTE) is an important research area in which small-diameter (<5 mm) tissue-engineered vascular grafts (TEVGs) are tried to be formed by combining cells and natural and/or synthetic scaffolds and generating tubular constructs. Pericytes are one of the main residents of both small and large blood vessels. They provide the stability of vessels by encircling ECs. They also have capacity to differentiate into cells that form blood vessels. These properties of PCs make them promising cell sources of VTE and TEVGs. There are several studies that include pericytes used in VTE and they are listed in the Table 1. He et al. isolated PCs from human skeletal muscle and obtained 3D vascular graft by seeding PCs on to elastomeric, tubular and porous scaffold [98]. They cultured this vascular graft in spinner flask bioreactor and implanted it to rat aorta. After 8 weeks, when the vascular graft was examined, the existence of smooth muscle and EC sheets were demonstrated.

Conclusion

Recent studies pointed out that PCs are the perivascular origin of MSCs. They have similar properties with MSCs like multipotential differentiation capacity, expression of MSC markers, lack of hematopoietic and EC markers, immunomodulation and supporting HSCs in BM. Beside all of these properties, they have crucial roles in blood vessel development, maturation and maintenance, angiogenesis, blood flow regulation and blood pressure control. These entire characteristics make PCs an attractive cell source for tissue engineering applications. As being natural residents of blood vessels and participants of blood vessel associated events, PCs may become preferred cells in vascular tissue engineering especially for constructing vascular grafts. In addition, studies about the usage of PCs in regenerative medicine and tissue engineering do not include the application of cells or tissue constructs to human models. There has been no clinical application of PCs or tissue construct made of them yet. So additional studies including PC-based vascular grafts may improve the area of vascular tissue engineering and open the way of clinical applications of PCs.

Conflict of Interest The authors declare no potential conflicts of interest.

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